REMARKS

Supplemental Material:

Applicants have provided additional material with this Amendment and Response in the form of a 37 CFR 1.132 Declaration. The material consists of experiments performed the process described in the instant application. The experiments show delivery of a therapeutic polynucleotides encoding VEGF (vascular endothelial growth factor) and EPO (erythropoietin), i.e. polynucleotides other than DNA encoding a marker protein, to limb skeletal muscle cells. The experiments introduce no new matter.

Objections to the claims:

The Examiner has requested that "around the limb" be removed from claim 35. In the Action dated 05/23/03, the Examiner stated that claims 34-36 were unclear because "a tourniquet or cuff is not 'applied' 'over the skin' and that "The limitation of applying pressure to the limb epidermis (claim 1 and 39) is not the same scope as applying a cuff of sphygmomanometer to the skin round the limb." Both "applied over the skin" and "around the limb" are supported in the specification on page 3 lines 8-11, page 5 lines 7-8 and 13-24, page 23 lines 22-24, page 25 lines 19-21 and page 32 lines 22-25.

Applicants have amended claim 39 to include "of the mammal" after "a blood vessel in a limb" as requested by the examiner. Applicants have amended claim 39 to make the step of "applying pressure to the limb..." comply with the Examiner's request. Applicants have amended claim 39 to include "of the limb" after "skeletal muscle cells" as requested by the examiner. Applicants have amended claim 39 to include "in the skeletal muscle cells" after "expressing the polynucleotide" as requested by the examiner.

Rejection of the claims under 35 USC §112:

Claim 1 has been rejected because the phrase "immunosuppressive treatment is new matter. Applicants respectfully disagree. Support for the claim can be found on page 3 lines 26-32, example 5 starting on page 28, example 8 on page 31, and example 9 starting on page 31. However, Applicants have amended the claims to recite "administering immunosuppressive drugs" and is support in the above mentioned section of the specification.

Claims 11 and 12 have been rejected because the terms "superficialis" and "profundus" are new matter. Applicants respectfully disagree. The abbreviations for the terms "superficialis" and "profundis", "spf." and "prof" respectively, can be found in the specification on page 26. The abbreviations were cited in the claims as originally filed. However, the Examiner stated in an Office Action dated 03/11/2003 that the abbreviated terms were ambiguous. Therefore Applicants amended the claims to cite the unabbreviated terms at the request of the Examiner. In a sister application (09/707,000) the same rejection was withdrawn in an action dated 02/18/2004. The Examiner has stated that "it is readily apparent that flexor digitorum spf. and flexor digitorum prof. must have referred to flexor digitorum superficialis and flexor digitorum profundis. No other flexor digitorum muscles could have such abbreviations."

Claims 34 and 35 have been rejected because the limitation of applying a tourniquet (34) or cuff (35) "around the limb" is new matter. Applicants respectfully disagree. Clear support for applying a tourniquet or cuff around a limb can be found in the specification on page 5 lines 5-11 and 13-24, page 23 lines 22-24, page 25 lines 19-21 and page 32 lines 22-24.

Claim 39 has been rejected because "impeding 'blood flow to the limb'" is new matter. The action states that the specification does not discuss anywhere, "impeding blood flow to the limb." Applicants respectfully disagree. Clear support can be found on page 3 lines 1-11 and lines 23-24, page 5 lines 5-11, page 23 lines 22-25, and page 31 lines 7-9. In addition, the use of a tourniquet and a sphygmomanometer are described, both of which are well known in the art to prevent blood flow to and from a limb or extremity.

Claim 39 has been rejected because the phrase "distal to" is new matter. Applicants respectfully disagree. Clear support for delivery to muscle cells distal to the occlusion is found on page 23 lines 22-24 and on page 32 lines 11-13 and 18-20. The action states that expression of the polynucleotide in muscle cells "distal to" and delivery of the polynucleotide to muscle cells "distal to" are not the same. However, it is not possible to have expression of a polynucleotide encoding a transgene in cells to which the polynucleotide has not been delivered. Nevertheless, a clear connection between delivery and expression is provided in the specification on page 5 lines 5-11, page 14 line 32 to page 15 line 2 and page 18 lines 9-10.

Claim 39 has been rejected because the phrase "does not diminish the use of the limb by the mammal" is new matter. Applicants respectfully disagree. Support can be found in the specification on page 3 line 13-19, page 22 lines 15-27, page 25 lines 17-25 and page 28 lines 6-23. The action states that the specification provides support for "a procedure that does not diminish the use of the limb by the mammal after the procedure." It is the Applicants' opinion that this statement is not materially different from "where inserting the polynucleotide, applying pressure, and expressing the polynucleotide (i.e. the procedure) does not diminish use of the limb by the mammal."

Claim 41 has been rejected because the phrase "a single injection" is new matter. Applicants have amended the claim to recite "administering immunosuprressive drugs within one day of injecting the polynucleotide." Support for the amendment can be found in the specification of page 3 lines 26-32, page 28 line 27 to page 29 line 5 and page 31 lines 13-15.

Claims 1-3, 5-7, 11, 12, 16-20, 24, 25, 27-31, 34-36, and 38-42 have been rejected under 35 U.S.C. 112, first paragraph. Applicants note that claims 5 and 27 were previously canceled. The action states that the specification does not reasonably provide enablement for expressing protein in skeletal muscle cells by occluding any limb, injecting any blood vessel and delivering the DNA to any skeletal muscle cell. Applicants believe the claims provide clear linkage between the limb, blood vessel and muscle cells. Claim 1 recites an *in vivo* process. The preample recites delivery of a polynucleotide to a limb skeletal muscle cell. Step a) recites injecting the polynucleotide into a blood vessel of the limb. Step b) recites impeding blood flow through the blood vessel. Claim 39, in step c) states that the polynucleotide is delivered to mammalian skeletal muscle cells distal to the applied pressure. Therefore, the claim does not encompass delivering a polynucleotide to any mammalian skeletal muscle cell as claimed by the examiner. Applicants have amended the claims to further clarify the linkage between the limb, blood vessel, and the muscle cell.

The action states, on pages 5-6, that Miller, Deonarain, Verma and Crystal establish the ability to target desired tissues was unpredictable. The instant application was filed on November 6, 2000 and is related to a provisional filed November 14, 1999. Miller was published in 1995, Deonarain in 1998, Verma in 1997, and Crystal in 1995. However, none of these publications contemplates the process taught by the Applicants in the instant application. Applicants do not find it reasonable to state that no progress can have been made

and that prior art documents which do not teach or contemplate the process taught by the Applicants can be given sufficient weight to indicate the Applicants' process can not work. The Applicants' process is clearly shown to be able to deliver polynucleotides to limb skeletal muscle cells. Furthermore, the Examiner acknowledged that the Applicants' process is enabling for "a method comprising applying a tourniquet to the limb of a mammal such that blood flow of a blood vessel in the limb is occluded and administering naked DNA to said blood vessel, wherein said DNA comprises a nucleic acid sequence encoding a marker protein operably linked to a promoter and wherein said marker protein is expressed to detectable levels in muscles of said limb" (page 4). The action also states that "The specification teaches administering naked plasmid DNA encoding a marker protein operably linked to a promoter to an artery of the arm or leg and obtaining expression in muscles cells of the arm or leg, respectively" (page 6).

The method taught by Milas is fundamentally different than the method taught by the Applicants. Milas taught perfusing adenovirus through the vasculature of an isolated limb using two catheters, one in the artery and one in the vein. Perfusion, with outflow, is required by Milas (paragraph 1 on page 2202). In contrast, Applicants' claims require only injection into a blood vessel, which inherently results in no outflow. "Resultant brisk outflow" as taught by Milas is equivalent to not impeding blood flow as taught by the Applicants. Failure to impede blood flow, as taught by the Applicants, results in failure to efficiently deliver polynucleotides to skeletal muscle cells, page 30 lines 23-24. Therefore, it is not unexpected that the method taught by Milas provides different results than the method taught by the Applicants.

The action states that the specification does not teach how to overcome the teachings of Ye. Ye taught a method that is fundamentally different than the method taught by the Applicants. Ye taught injection of adenovirus into the retro-orbital venous plexus, a vessel of the eye. In contrast, Applicants teach injection of a polynucleotide into a blood vessel in a limb. Ye taught clamping of the hepatic artery and the portal vein to prevent blood circulation, and thus the adenovirus, from reaching the liver. In contract, Applicants teach the use of a cuff to impede blood flow to a limb. Therefore, it is not unexpected that the method taught by Ye provides different results than the method taught by the Applicants.

The action states, on page 7 and 9 that it can not be determined how the delivery of DNA to the specific muscles of the arm and leg is effected by the location of the blood vessel injected, the type of polynucleotide, the method of occlusion (claims 11, 12, 16, 17, 24, 25, 29-31). The specification states that the injected polynucleotides are delivered to all muscle groups distal to the occlusion. Support for delivery of polynucleotides to muscle cells in the limb distal to the occlusion can be found on page 32 lines 18-19. Support for delivery to all muscle groups in the limb distal to the occlusion can be found in the examples. For injection into rhesus monkey, injection and occlusion were located just above the elbow or knee (example 1). Delivery, as evidenced by luciferase expression, was then observed in muscles throughout the lower limb (example 3). In animals in which the occlusion was located slightly higher on the hind limb, polynucleotide delivery was observed in muscles of the upper leg as well as the lower leg and foot (example 10, see especially page 32 lines 18-19). Delivery to each of the named muscle groups is explicitly shown in table 1 in example 3 (page 26-28). For delivery to any of the muscle groups listed in claims 11, 12, 16, 17, 24, 25 or 29-31, the muscle group must simply be located distal to the tourniquet.

The action states that the specification does not correlate the results obtained with naked plasmid DNA to any other vector (e.g. adenovirus). Applicants respectfully disagree. The supplemental information provided with the amendment filed by the Applicants on May 9, 2003 demonstrated that the method could be used to delivery viral vectors (adenovirus) and non-viral vectors (polycation/DNA complexes) to limb skeletal muscle cells.

The action states that the specification does not provide guidance to determine why or when an immunosuppressive agent is administered. Applicants respectfully disagree. Page 3 lines 27-28 and Example 5, starting on page 28, describe when immunosuppression drugs may be administered. Example 5 further shows that in animals receiving immunosuppression, expression of the delivered gene persists for a longer period of time.

The action states, on page 8, that the specification only enables delivering DNA encoding a marker protein operable linked to a promoter. Applicants respectfully disagree. A gene encoding a therapeutic gene, or a gene whose effect on a cell is being investigated, is functionally equivalent to a marker gene for the purposes of delivery using the described invention; see page 9 lines 13-30. In addition, delivery of the therapeutic gene, human factor IX, to limb skeletal muscle cells is shown in example 8 on page 31. While delivery in this

example is performed without a cuff, the example demonstrates the equivalence of polynucleotides encoding marker genes and therapeutic genes (or other genes of research or intellectual interest). As further evidence of enablement of delivery of polynucleotides encoding therapeutic protein, Applicants submit Supplemental Information showing delivery of polynucleotides encoding VEGF and EPO. The delivery of the polynucleotides was done using the same method as described in the specification.

The action states on page 10 that the supplemental information filed with the declaration on 5-9-03, does not correlate to the teachings in the specification and provides more teachings than were provided in the specification. Applicants respectfully disagree. The instant specification teaches that the method can be combined with "a pharmaceutical or biologically-active agent (such as papaverine) to increase vascular permeability" (page 5 lines 26-28). The specification, on page 16 line 28 to page 17 line 7, provides further support for the use of papaverine and collagenase ("an enzyme could digest the extracellular material"). The use of papaverine is also used in examples 1 and 8. Injection of polynucleotide in a 10 ml solution in 10 sec is found in example 8 in the specification. Further support for the volumes and rates used in the previously filed declaration can be found in the specification on page 17 lines 9 to page 18 line 6.

The action states, on pages 10-11, that the polynucleotide must encode a protein operably linked to a promoter for expression to occur. This statement is incorrect. The polynucleotide can encode an RNA molecule which is not translated into protein but which has a cellular function itself.

The action states, on page 11, that claim 1 is newly indefinite. Applicants have amended the claim to remove the indefiniteness.

Claims 34 and 35 have been amended to contain terms with proper antecedent basis.

Claim 38 has been canceled.

Claim 39 has been amended to supply parallel language as requested by the Examiner.

The action states that the term distal in the claim is unclear. Applicants respectfully disagree. The term distal is a standard term used in the art. Distal means anatomically situated away from the origin or point of attachment, or from the center (midline or mesial plane) of the body.

Claim 39 has been amended to more clearly set forth that the polynucleotides are delivered to and expressed in skeletal muscle cells in the limb.

Claim 39 has been rejected (page 16 of the Action) because the final phrase, "wherein ...by the mammal." is unclear. Applicants have amended the claim to recite " ... subsequent use of the limb...." Support for the amendment can be found in the specification on page 3 line 13-19 and page 25 lines 17-25. The phrase "does not diminish subsequent use of the limb" encompasses both the functional parameters of the limb and the frequency of use of the limb.

Claim 40 has been amended to recite repetitive administration of immunosuppressive drugs. Support for the amendment can be found in the specification on page 3 line 26 to page 4 line 2, in example 5 starting on page 28, in example 8 on page 31, and in example 9 starting on page 31.

Rejection of the claims under 35 USC §102:

The claims have been rejected under 35 U.S.C. 102 as being anticipated by Draijer-van der Kaaden (U.S. Patent 6,495,131). Draijer-van der Kaaden et al and Milas et al both teach the same limb perfusion technique, i.e. cunnulation of both the femoral artery and vein, and both reference the same papers for the technique of isolated limb perfusion (Benckhuysen et al. 1982 and Manusama et al. 1996). For the reasons stated above in response to the 112 rejections, it is the Applicants' opinion that the method taught by Draijer-van der Kaaden et al. (and Milas et al.) is fundamentally different from the method taught by the Applicants.

The claims have been rejected under 35 U.S.C. 102 as being anticipated by Milas et al. (1997). The action states that "The DNA of Milas was inherently delivered to skeletal muscle cells because occlusion of the femoral artery using a tourniquet results in delivery of DNA to skeletal muscle and is the same method used by applicants in example 10." As discussed above, the method taught by Milas is different than the method taught by the Applicants. Furthermore, Milas directly states that, using their method, "... no β-gal staining was

apparent in the muscular tissues of the perfused limb." indicating no delivery of DNA to skeletal muscle cells (page 2201, second column, first full paragraph). Applicants have amended the claims to require expression in skeletal muscle cells. Support from the amendment can be found page 5 lines 5-7, page 9 lines 13-16, page 14 line 32 to page 15 line 2, example 2 starting on page 24, example 3 starting on page 25, example 6 on page 30 and example 9 starting on page 31. The action states that the presence of small inflammatory cell infiltrates are an indication that the DNA had inherently been delivered "to the limb skeletal muscle cells" in the method taught by Milas. Applicants can find no indication in the publication by Milas which indicates that the small inflammatory cell infiltrates (presence of immune system cells) provides evidence that DNA was delivered to skeletal muscle cells.

Applicants have amended claims 1 and 39 to specify that the pressure applied against the skin of the limb is applied non-invasively. Support for the amendment can be found on page 5 lines 13-24. The amendment distinguishes the method taught by the Applicants from the method taught by Milas et al.

The claims have been rejected under 35 U.S.C. 102 as being anticipated by Von der Leyen et al. 1999). Applicants previously argued that Von der Leyen did not observe delivery of polynucleotides to skeletal muscle cells. The Examiner states, and the Applicants agree, that Von der Leyen did not test for delivery to skeletal muscle. Nevertheless, the Examiner argues that the method of Von der Leyen inherently results in expression in skeletal muscle cells and state that Von der Leyen forced DNA through the "blood vessel" wall (as evidenced by pg. 2362, col. 1, line 14; the "blood vessel" wall was incorrectly called the "muscle" wall in the action). The term "through" in this instance refers to access to all the layers of the blood vessel (see next sentence in von der Leyen et al), and not to transport of molecules completely out of the blood vessel. Furthermore, Von der Leyen placed a protective sheath directly around the vessel (pg. 2356, col. 2, transfection procedure, first sentence; and pg. 2362, col. 1, second full paragraph). This sheath, placed around the isolated artery at physiological diameter, would inherently prevent movement of DNA out of the vessel and into the surrounding skeletal muscle as the Examiner supposes.

The Action states that the reason Von der Leyen used the sphygmomanometer is irrelevant. Applicants disagree. Von der Leyen used a sphygmomanometer, and a PTCA manometer, not to cause external pressure on a blood vessel and thus occlude blood flow, but to monitor

pressure of a solution. Von der Leyen writes, "The transfection solution was applied under a pressure of... . For pressures between 0 and 300 mmHg, a commercially available sphygmomanometer was used to monitor pressure" page 2357 bridging to page 2357.

Claim Rejections under 35 USC 103:

The claims have been rejected under 35 U.S.C. 103 as being unpatentable over Budker 1998 in view of Milas 1997. The action states that: a) the limitation of applying transient immunosuppression is equivalent to temporarily occluding blood vessels; b) the limitation of applying continuous immunosuppression is taught by Milas; and, c) the metes and bounds of continuous and transient immunosuppression is unclear. Applicants point out that the limitations of transient and continuous immunosuppression are not present in the claims. Also, neither temporarily occluding blood vessels nor administering collagenase constitutes immunosuppression. Neither activity removes cells of the immune system from the limb or diminishes the ability of immune cells present in the limb from carrying out their function.

Applicants have amended claims 1 and 39 to include the limitation that blood flow to and from the limb is impeded. Applicants have also amended the claims to recite the limitation that the blood flow is impeded by a non-invasive device. Support for impeding blood flow to and from the limb can be found on page 3 lines 1-11, page 5 lines 5-11, page 23 lines 22-25, and page 31 lines 7-9. Support for a non-invasive device can be found on page 5 lines 13-24. Milas did not teach a non-invasive method for occluding blood flow to and from a limb. Milas taught an invasive placement of the tourniquet underneath the inguinal ligament (Fig. 1 legend; Fig. 2; and pg. 2198 col. 2, "Operative Technique, end of paragraph). Milas also explicitly taught that blood flow from the limb was not impeded (Fig. 1; pg. 2199, col. 1., lines 8-13; and pg. 2202, col. 1). Milas additionally taught ligation of the artery and vein distal to the cannulation sites after the injection with their method. It is the Applicants' opinion that the amendments to the claims, taken together with the Applicants' arguments, obviate the rejection.

The claims have been rejected under 35 U.S.C. as being unpatentable over Milas (1997) in view of Nabel (1999). Applicants agree that Nabel taught pre-treating with an immunosuppressant. Applicants disagree with the Examiner's statement that "adenovirus was inherently delivered to skeletal muscle cells as claimed because the method of Milas is identical to that used by the applicants in example 10." As presented above in response to the

112 rejections, it is the Applicants' opinion that the method taught by Milas is fundamentally different from the method taught by the Applicants. Milas taught that isolation of a limb with an invasive tourniquet, cannulation of both the femoral artery and vein, and perfusion of a limb with brisk outflow using a solution containing adenovirus failed to delivery polynucleotides to limb skeletal muscle cells. A combination of the method taught by Milas with the method taught by Nabel would still fail to deliver DNA to skeletal muscle cells since neither Milas or Nabel teach how to deliver polynucleotides to skeletal muscle cells. There is no suggestion or motivation provided in either Milas or Nabel that would indicate that addition of an immunosuppressant drug would alter the target cells and enable adenovirus delivery to skeletal muscle cells using the method of Milas.

The claims have been rejected under 35 U.S.C. as being unpatentable over Wolff (2001) in view of Milas (1997). As discussed above, Milas taught that isolation of a limb with an invasive tourniquet, cannulation of both the femoral artery and vein, and perfusion of a limb with brisk outflow using a solution containing adenovirus failed to delivery polynucleotides to limb skeletal muscle cells. Milas also taught an invasive tourniquet and taught away from impeding blood flow from the limb. Thus the tourniquet taught by Milas does not contain all of the limitation set forth in the Applicants' claims. It is the Applicants' opinion that the amendments to the claims, taken together with the Applicants' arguments, obviate the rejection.

Obviousness-type Double Patenting Rejection:

The claims have been rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over Wolff (U.S. patent 6,456,387) in view of Milas. Wolff did not, in example 8 in column 17 as stated in the action, deliver naked plasmid DNA to a clamped femoral artery and obtain expression in the liver. '387 did teach, in example 18, delivery of polynucleotide to skeletal muscle. However, '387 did not teach or contemplate applying non-invasive external pressure against the skin of the limb such that blood flow to and from the limb is impeded. This novel approach allows for the non-surgical delivery of polynucleotides to limb skeletal muscle cells, which is not possible with the application of clamps directly on vessels as described in '387. As discussed above, Milas taught that isolation of a limb with a tourniquet, cannulation of both the femoral artery and vein, and perfusion of a limb with brisk outflow using a solution containing adenovirus failed to delivery polynucleotides to limb skeletal muscle cells. Milas also taught an invasive

tourniquet and taught away from impeding blood flow from the limb. Thus the tourniquet taught by Milas does not contain all of the limitations set forth in the Applicants' claims. It is the Applicants' opinion that the amendments to the claims, taken together with the Applicants' arguments, obviate the rejection.

The claims have been rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over U.S. patent 6,627,616. '616 did not teach or contemplate applying non-invasive external pressure against the skin of the limb such that blood flow to and from the limb is impeded. This novel approach allows for the non-surgical delivery of polynucleotides to limb skeletal muscle cells, which is not possible with the application of clamps directly on vessels as described in '616.

The Examiner's objections and rejections are now believed to be overcome by this response to the Office Action. In view of Applicants' amendment and arguments, it is submitted that claims 1-3, 6, 7, 11, 12, 16-20, 24, 25, 28-31, 34-36, and 39-42 should be allowable.

Respectfully submitted,

Mark K. Johnson Reg. No. 35,909
Mirus
505 South Rosa Road
Madison, WI 53719
608-238-4400

I hereby certify that this correspondence is being sent by facsimile transmission to: Commissioner for Patents, PO Box 1450, Alexandria, VA 22313-1450 on this date: 4/16/04

Kirk Ekena